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Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments

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Abstract

A survey of fresh and minimally-processed fruit and vegetables, and sprouts was conducted in several retail establishments in the Lleida area (Catalonia, Spain) during 2005–2006 to determine whether microbial contamination, and in particular potentially pathogenic bacteria, was present under these commodities. A total of 300 samples—including 21 ready-to-eat fruits, 28 whole fresh vegetables, 15 sprout samples and 237 ready-to-eat salads containing from one to six vegetables—were purchased from 4 supermarkets. They were tested for mesophilic and psychrotrophic aerobic counts, yeasts and moulds, lactic acid bacteria, Enterobacteriaceae, presumptive *E. coli* and *Listeria monocytogenes* counts as well as for the presence of *Salmonella*, *E. coli* O157:H7, *Yersinia enterocolitica* and thermotolerant *Campylobacter*.

Results for the fresh-cut vegetables that we analyzed showed that, in general, the highest microorganism counts were associated with grated carrot, arugula and spinach (7.8, 7.5 and 7.4 log cfu g^{-1} of aerobic mesophilic microorganisms; 6.1, 5.8 and 5.2 log cfu g^{-1} of yeast and moulds; 5.9, 4.0 and 5.1 log cfu g^{-1} lactic acid bacteria and 6.2, 5.3 and 6.0 log cfu g^{-1} of Enterobacteriaceae). The lowest counts were generally associated with fresh-cut endive and lettuce (6.2 and 6.3 log cfu g^{-1} of aerobic mesophilic microorganisms; 4.4 and 4.6 log cfu g^{-1} of yeast and moulds; 2.7 and 3.8 log cfu g^{-1} lactic acid bacteria and 4.8 and 4.4 log cfu g^{-1} of Enterobacteriaceae). Counts of psychrotrophic microorganisms were as high as those of mesophilic microorganisms. Microbiological counts for fresh-cut fruit were very low. Sprouts were highly contaminated with mesophilic (7.9 log cfu g^{-1}), psychrotrophic microorganisms (7.3 log cfu g^{-1}) and Enterobacteriaceae (7.2 log cfu g^{-1}) and showed a high incidence of *E. coli* (40% of samples). Of the samples analyzed, four (1.3%) were *Salmonella* positive and two (0.7%) harboured *L. monocytogenes*. None of the samples was positive for *E. coli* O157:H7, pathogenic *Y. enterocolitica* or thermotolerant *Campylobacter*.

Keywords: Incidence; Foodborne pathogens; Fresh-cut; Ready-to-eat; *Salmonella*; *E. coli*; *L. monocytogenes*; *Campylobacter*; *Y. enterocolitica*

1. Introduction

Fresh fruit and vegetables are essential components of the human diet and there is considerable evidence of the health and nutritional benefits associated with the consumption of fresh fruit and vegetables. In the USA, Canada, New Zealand and several European Union states, public health institutions have run campaigns recommending the daily consumption of at least five daily servings of fruit and vegetables. As well as a significant rise in the consumption of fresh produce for health benefits, there have also been significant changes in lifestyles and major shifts in consumption trends. These changes have produced a demand for a wider range of products, and have led people to spend less time cooking at home and to eat out more often. Such trends have been reflected in an increase in the popularity of salad bars and have prompted the appearance of minimally-processed convenience foods that are ready-to-eat. Among them, the consumption of fresh-cut or minimally-processed fruit and vegetables has undergone a sharp increase. Consumption of fresh-cut fruits and vegetables in Spain is still low (1–1.5 kg per person per year) compared with the rest of Europe (UK, 12 kg; France, 6 kg; Italy, 4 kg and Germany, Belgium and Netherlands with more than 3 kg) and USA (30 kg per person). However, in Spain this market is showing an annual increase of sales of about 20%, with 53,465 t sold in 2006 (Anonymous, 2007).

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Fresh produce can be a vehicle for the transmission of bacterial, parasitic and viral pathogens capable of causing human illness and a number of reports refer to raw vegetables harbouring potential foodborne pathogens (Nguyen-the and Carlin, 1994; Beuchat, 1996). *Listeria monocytogenes* (Schlech et al., 1983), *Salmonella* (Doyle, 1990), and *Escherichia coli* (Nguyen-the and Carlin, 1994) have been isolated from raw vegetables, which can become contaminated while growing or during harvesting, postharvest handling, or distribution. The incidence of foodborne outbreaks caused by contaminated fresh fruit and vegetables has increased in recent years (Mukherjee et al., 2006). The pathogens most frequently linked to produce-related outbreaks include bacteria (*Salmonella*, *E. coli*), viruses (Norwalk-like, hepatitis A), and parasites (*Cryptosporidium*, *Cyclospora*) (Tauxe et al., 1997), with *Salmonella* and *E. coli* O157:H7 being the leading causes of produce-related outbreaks in the USA (Olsen et al., 2000). Fresh produce and sprouts have been implicated in a number of documented outbreaks of illness in countries such as Japan (Nat'l. Inst. Inf. Dis., 1997; Gutierrez, 1997), the USA (De Roever, 1998) and EU (Emberland et al., 2007; Nygård et al., 2004; Pezzoli et al., 2007; PHLS, 2000; Söderström et al., 2005). In September 2006, an *E. coli* O157:H7 outbreak affected 26 US States which involved about 200 cases of illness, including some of Hemolytic Uremic Syndrome (HUS) and resulted in three deaths (FDA, 2006). Data indicated that fresh spinach grown in three Californian counties was the source of the bacterium. Fresh fruit and vegetables may therefore pose a food safety risk because they are consumed raw and are susceptible to be contaminated by fecal material and soil on the farm (Mukherjee et al., 2004).

Several national microbiological guidelines have been published for ready-to-eat food in countries such as: the UK (PHLS, 2000), Spain (Real Decreto 3484/2000); France (Nguyen-the and Carlin, 1994), Germany (Lund, 1993) and Japan (FEHD, 2002). The European Commission has also recently published a new regulation (n° 2073/2005—Official Journal of the European Union, 2005, L 338) that establishes a common food safety and process hygiene criteria for food in EU countries, and which covers pre-cut fruit and vegetables and sprouts.

Although there have been a number of reports about microbiological contamination involving whole fresh produce (García-Villanova et al., 1987a,b; Johannessen et al., 2002; McMahon and Wilson, 2001; Salleh et al., 2003; Johnston et al., 2005; Mukherjee et al., 2004, 2006; FDA, 2001, 2003), there is still little information about microbial contamination of fresh-cut fruit and vegetables (FEHD, 2002; Sagoo et al., 2003, Tournas, 2005, Tournas et al., 2006) and sprouts (Robertson et al., 2002) and even less relating to the EU. A report by the EU Scientific Committee on Food (2002) stated that the prevalence of foodborne pathogens on fruit and vegetables and their involvement in outbreaks are not well documented from a European perspective.

The aim of this study was therefore to investigate the microbiological quality of fresh and minimally-processed fruit and vegetables, and sprouts commercialized in Spain and the incidence of the main bacterial foodborne pathogens in this area.

2. Materials and methods

2.1. Samples

A total of 300 samples of whole vegetables, minimally-processed (fresh-cut) fruit and vegetables and sprouts were analyzed during the period 2005–2006. The samples analyzed

Table 1
List of methodologies used to determine microbial quality

Determination	Methodology	Description
Aerobic mesophilic count	ISO 4833:2003	Microbiology of food and animal feeding stuffs—Horizontal methods for the enumeration of microorganisms. Colony-count technique at 30 °C.
Psychrotrophic microorganisms	ISO 17410:2001	Microbiology of food and animal feeding stuffs—Horizontal methods for the enumeration of psychrotrophic microorganisms.
Yeasts and moulds	ISO 7954:1987	Microbiology—General guidance for enumeration of yeasts and moulds—Colony count technique at 25 °C
Lactic acid bacteria	ISO 15214:1998	Microbiology of food and animal feeding stuffs—Horizontal methods for the enumeration of mesophilic lactic acid bacteria—Colony-count technique at 30 °C.
Enterobacteriaceae	ISO 21528-2:2004	Microbiology of food and animal feeding stuffs—Horizontal methods for the detection and enumeration of Enterobacteriaceae—Part 2: Colony-count method
Presumptive *E.coli*[a]	ISO 7251:2005	Microbiology of food and animal feeding stuffs—Horizontal methods for the detection and enumeration of presumptive *Escherichia coli*—Most probable number technique
Salmonella[b]	ISO 6579:2002	Microbiology of food and animal feeding stuffs—Horizontal methods for the detection of *Salmonella* spp.
L. monocytogenes	ISO 11290-2:1998	Microbiology of food and animal feeding stuffs—Horizontal methods for the detection and enumeration of *Listeria monocytogenes*. Part 2: Enumeration method.
Y. enterocolitica[c]	ISO 10273:2003	Microbiology of food and animal feeding stuffs—Horizontal methods for the detection of presumptive pathogenic *Yersinia enterocolitica*
Campylobacter	ISO 10272:1995	Microbiology of food and animal feeding stuffs—Horizontal methods for the detection of thermotolerant *Campylobacter*

[a]*E. coli* strains isolated were plated in Tergitol BCIG agar and Sorbitol MacConkey Agar and incubated at 44 ± 1 °C for the detection of β-glucuronidase and sorbitol positive strains, respectively.
[b]Presumptive *Salmonella* were sent to the 'National Reference Laboratory' ('Instituto de Salud Carlos III', Mahadahonda, Spain) for confirmation.
[c]Strains identified as *Y. enterocolitica* by biochemical tests were sent to the 'Laboratorio de Microbiología, Universidad de Navarra' in Spain to determine their pathogenicity.